

The Synergy of Green Tea and Penicillin G Against *Bacillus Subtilis*

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Bacterial samples of *Bacillus subtilis* were cultured on nutrient agar using Petri dishes, with three antibiotic discs (Penicillin G, 6µg) on each surface. Agar samples had been prepared by agar dilution (Wiegand et al., 2008) using the green tea extract samples of the respective concentrations (agar: tea extract 3:1); there were three Petri dishes per concentration. The dishes were sealed and incubated at 30°C for 24 hours. Following incubation, the area of each inhibition zone was estimated. Inhibition area was plotted against green tea concentration. Strength of association was measured using Pearson's correlation. A linear regression line was fitted to the data, and a statistical test based on the t-distribution (Bland, 1995) was applied to determine whether the gradient observed was statistically significant.

Green tea steeping time and temperature varies with different tea. The hottest steeping temperatures are 81 to 87 °C (178 to 189 °F) water and the longest steeping times two to three minutes. The coolest brewing temperatures are 61 to 69 °C (142 to 156 °F) and the shortest times about 30 seconds. In general, lower-quality green teas are steeped hotter and longer, whereas higher-quality teas are steeped cooler and shorter. Steeping green tea too hot or too long will result in a bitter, [astringent](#) brew, regardless of the initial quality, because it will result in the release of an excessive amount of [tannins](#). High-quality green teas can be and usually are steeped multiple times; two or three steepings is typical. The steeping technique also plays a very important role in avoiding the tea developing an overcooked taste. The container in which the tea is steeped or teapot should also be warmed beforehand so that the tea does not immediately cool down. It is common practice for tea leaf to be left in the cup or pot and for hot water to be added as the tea is drunk until the flavor degrades.

Green tea contains a variety of enzymes, amino acids, carbohydrates, lipids, sterols, related compounds, dietary minerals, and phytochemicals such as polyphenols, flavanols, and caffeine. Polyphenols found in green tea include but are not limited to epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate, and epicatechin; flavanols such as kaempferol, quercetin, and myricetin are also found in green tea. Numerous claims have been made for the health benefits of green tea based on its chemical composition, in vitro studies, animal studies, and human epidemiological studies. Preliminary research on many of these claims is promising, but many also require further study to evaluate.

In 2011 a panel of scientists published a report on green tea's claimed health effects at the request of the European commission: in general they found that the claims made for green tea were not supported by good scientific evidence. Although the mean content of flavonoids and catechins in a cup of green tea is higher than that in the same volume of other food and drink items that are traditionally considered to promote health, flavonoids and catechins have no proven biological effect in humans.

Bacillus subtilis, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. A member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. *B. subtilis* has historically been classified as an obligate aerobe, though evidence exists that it is a facultative aerobe. *B. subtilis* is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation. It is one of the bacterial champions in secreted enzyme production and used on an industrial scale by biotechnology companies.

Bacillus subtilis is a Gram-positive bacterium, rod-shaped and catalase-positive. It was originally named *Vibrio subtilis* by Christian Gottfried Ehrenberg, and renamed *Bacillus subtilis* by Ferdinand Cohn in 1872 (*subtilis* being the Latin for 'fine'). *B. subtilis* cells are typically rod-shaped, and are about 4-10 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of about 4.6 fL at stationary phase. As other members of the genus *Bacillus*, it can form an endospore, to survive extreme environmental conditions of temperature and desiccation.[6] *B. subtilis* is a facultative anaerobe and had been considered as an obligate aerobe until 1998. *B. subtilis* is heavily flagellated, which gives it the ability to move quickly in liquids. *B. subtilis* has proven highly amenable to genetic manipulation, and has become widely adopted as a model organism for laboratory studies, especially of sporulation, which is a simplified example of cellular differentiation. In terms of popularity as a laboratory model organism, *B. subtilis* is often considered as the Gram-positive equivalent of *Escherichia coli*, an extensively studied Gram-negative bacterium.

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